EDGEWOOD CHEMICAL BIOLOGICAL CENTER
U.S. ARMY RESEARCH, DEVELOPMENT AND ENGINEERING COMMAND
Aberdeen Proving Ground, MD 21010-5424

ECBC-TR-1341

SEPARATION OF VX, RVX, AND GB ENANTIOMERS USING LIQUID CHROMATOGRAPHY–TIME-OF-FLIGHT MASS SPECTROMETRY

Sue Y. Bae
Mark D. Winemiller

RESEARCH AND TECHNOLOGY DIRECTORATE

February 2016

Disclaimer

REPORT DOCUMENTATION PAGE	*Form Approved* *OMB No. 0704-0188*

1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED** *(From - To)*
XX-02-2016	Final	Mar 2015 – Aug 2015

4. TITLE AND SUBTITLE
Separation of VX, RVX, and GB Enantiomers Using Liquid Chromatography–Time-of-Flight Mass Spectrometry

5a. CONTRACT NUMBER

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Bae, Sue Y.; and Winemiller, Mark D.

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Director, ECBC, ATTN: RDCB-DRC-C, APG, MD 21010-5424

8. PERFORMING ORGANIZATION REPORT NUMBER
ECBC-TR-1341

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; distribution is unlimited.

13. SUPPLEMENTARY NOTES

14. ABSTRACT:
Chemical nerve agents such as VX (*O*-ethyl *S*-[2-(diisopropylamino)ethyl] methylphosphonothioate), RVX (*S*-[2-(diethylamino)ethyl] *O*-isobutyl-methylphosphonothioate), and GB (isopropyl methylphosphonofluoridate) exist as a mixture of two enantiomers. A Lux 5u Cellulose-1 normal-phase chiral liquid chromatography column (Phenomenex; Torrance, CA) was used to separate the enantiomers for all V and G agents within 15 min. Atmospheric pressure chemical ionization mode was used for liquid chromatography–time-of-flight mass spectrometry analysis. For large-scale separation and quantitation, the UV absorbance at 210 nm (with a bandwidth of 4 nm) was referenced to a wavelength of 360 nm. An Agilent series 1200 fraction collector (Agilent Technologies; Santa Clara, CA) was used to collect both (+) and (–) enantiomers for each chemical agent separated. Identification and isolation of each enantiomer of a chemical agent is very beneficial for in vitro and in vivo toxicological studies.

15. SUBJECT TERMS
O-ethyl *S*-[2-(diisopropylamino)ethyl] methylphosphonothioate (VX)
S-[2-(diethylamino)ethyl] *O*-isobutyl-methylphosphonothioate (RVX)
Isopropyl methylphosphonofluoridate (GB, sarin)
UV chromatography
Enantiomers

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON** Renu B. Rastogi
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			**19b. TELEPHONE NUMBER** *(include area code)*
U	U	U	UU	20	(410) 436-7545

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

Blank

PREFACE

This work was started in March 2015 and completed in August 2015.

The use of either trade or manufacturers' names in this report does not constitute an official endorsement of any commercial products. This report may not be cited for purposes of advertisement.

This report has been approved for public release.

Blank

CONTENTS

FIGURES

SEPARATION OF VX, RVX, AND GB ENANTIOMERS USING LIQUID CHROMATOGRAPHY–TIME-OF-FLIGHT MASS SPECTROMETRY

1. INTRODUCTION

Tetra-coordinate pentavalent phosphorus compounds have four σ bonds arranged through sp^3 hybrid orbitals as occurs in tetrahedral carbon compounds. The first enantiomeric phosphorus compound, ethylmethylphenylphosphine oxide, or Et(Me)P(O)Ph, was isolated in 1911 by J. Meisenheimer and L. Lichtenstadt.[1] Many organophosphorus (OP) pesticides have an asymmetric phosphorus atom, and several have been separated into individual enantiomers.[2] OP nerve agents such as *O*-ethyl *S*-[2-(diisopropylamino)ethyl] methylphosphonothioate (VX) have an asymmetric phosphorus atom, and synthesis of VX yields a racemic mix of two enantiomers, P(+) and P(–). Although the enantiomers have identical physical properties, their biological activities depend greatly on their chirality. Compared with the P(+) enantiomer, the P(–) enantiomer has an order of magnitude higher effect on the rate of inhibition of acetylcholinesterase, which lowers the LD_{50} (the dose that is lethal to 50% of test subjects) in mice.[3,4]

The literature is limited regarding the use of chromatographic techniques to separate the enantiomers of chemical warfare agents.[5–7] Although reports exist of VX being separated, the enantiomer separation took longer than 65 min with use of liquid chromatography (LC) and tandem mass spectrometry, and more than 5 h with use of gas chromatography and mass spectrometry (MS). Only one report, by J. Smith, described a baseline-resolved separation of the VX enantiomers in less than 10 min.[8] This was accomplished using a Chiralcel OD-H column (Daicel Corporation; Osaka-Shi, Japan). We now report the development of an analytical method for separating the enantiomers of nerve agents VX, *S*-(2-(diethylamino)ethyl) *O*-isobutyl-methylphosphonothioate (RVX), and isopropyl methylphosphonofluoridate (GB) (Figure 1) using a normal-phase chiral LC column and atmospheric pressure chemical ionization mass spectrometry (APCI–MS). This separation was then transferred to a preparative-scale instrument, and a UV detection source was used to collect the individual P(+) and P(–) enantiomers of the desired agents.

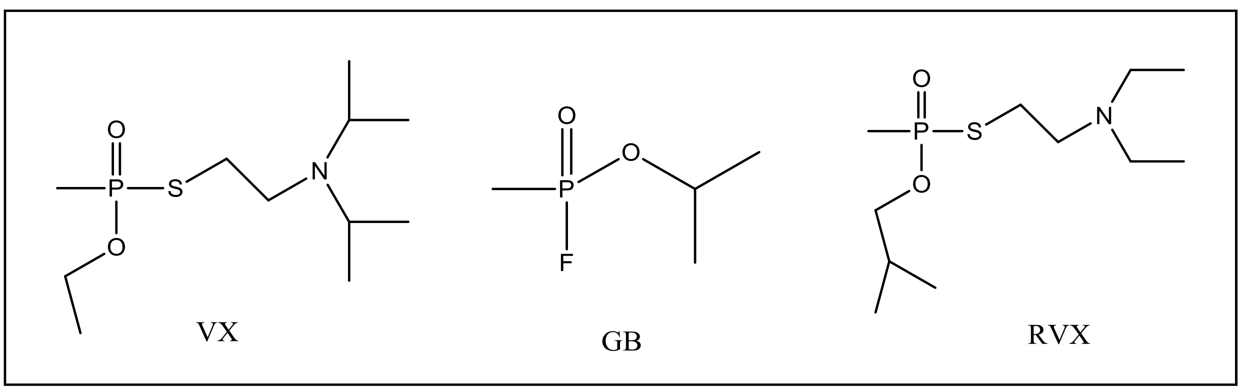

Figure 1. Chemical structures of three chemical warfare agents.

2. EXPERIMENTAL METHODS

2.1 Reagents and Chemicals

Nerve agents VX, RVX, and GB, all of which were of >95% purity, were synthesized by the Agent Chemistry Team from the Research and Technology Directorate of the U.S. Army Edgewood Chemical Biological Center (Aberdeen Proving Ground, MD). Samples were made at a 100 µg/mL level for analytical separation and a 22 mg/mL level for preparative separation. All reagents and solvents were high-performance LC grade. Hexane and isopropyl alcohol were purchased from Fisher Scientific (Waltham, MA).

2.2 Instrumentation

The analytical separations of the enantiomers were characterized using an Agilent 1200 Infinity series LC system (Agilent Technologies; Santa Clara, CA), and APCI–MS was performed on a Lux Cellulose-1 column (250 × 4.6 mm, 5 µm; Phenomenex; Torrance, CA). The mobile phase consisted of *n*-hexane (A) and isopropyl alcohol (B), and sample volume was 10 µL. Separation was achieved using isocratic conditions of 96/4 (v/v %) A/B for VX and RVX and 95/5 (v/v %) A/B for GB, with a flow rate of 0.6 mL/min.

The enantioselective preparative-scale separation of agents was achieved using an Agilent 1100 series preparative-scale LC system equipped with a diode array detector. Injections were monitored at 210 nm. Separation was achieved using a Phenomenex Lux Cellulose-1 Axia packed column (250 × 30 mm) with an isocratic condition of 96/4 (v/v %) A/B, a flow rate of 20 mL/min, and a sample volume of 1000 µL. Both VX enantiomers were baseline separated within 23 min. The Agilent 1200 Infinity series fraction collector was configured using the peak-time-based collection protocol, and the separated enantiomers were combined into 500 mL round-bottom flasks for solvent removal by rotary evaporation. Individual enantiomers were confirmed by polarimetry using a Vee Gee polarimeter (Vee Gee Scientific; Kirkland, WA) and a 10 mL optical cell.

3. RESULTS AND DISCUSSION

For the LC–MS analytical analysis, the MS system was operated in total ion chromatogram (TIC) mode at *m/z* 50–500 for VX, RVX, and GB. APCI mode was used for LC–time-of-flight MS. A Lux 5u Cellulose-1 column and normal-phase LC were used with a mobile phase of 96/4 (v/v %) hexane/isopropyl alcohol at a flow rate of 0.6 mL/min. The enantiomers were baseline-resolved within 15 min. The analytical separation method was then transferred to the preparative-scale LC for large-scale isolation of the desired enantiomers.

As shown in Figure 2, the VX enantiomers eluted at 9.5 and 11.3 min when the fragmentor voltage was 100 V. As expected, the mass spectrum for the enantiomer at 9.5 min was identical to that for the enantiomer at 11.3 min . A UV detector was incorporated in the analytical analysis to monitor for the appearance of VX. The observation wavelength was 210 nm using a bandwidth of 2 nm, and the reference wavelength was 300 nm. The analytical separation method was successfully transferred to the preparative-scale system using a Phenomenex Lux Cellulose-1 Axia packed column. The fraction collector was set as time-based, and fractions were collected into multiple test tubes from 13 to 17.5 min for the P(+) enantiomer and from 18 to 23.5 min for the P(–) enantiomer (Figure 3).

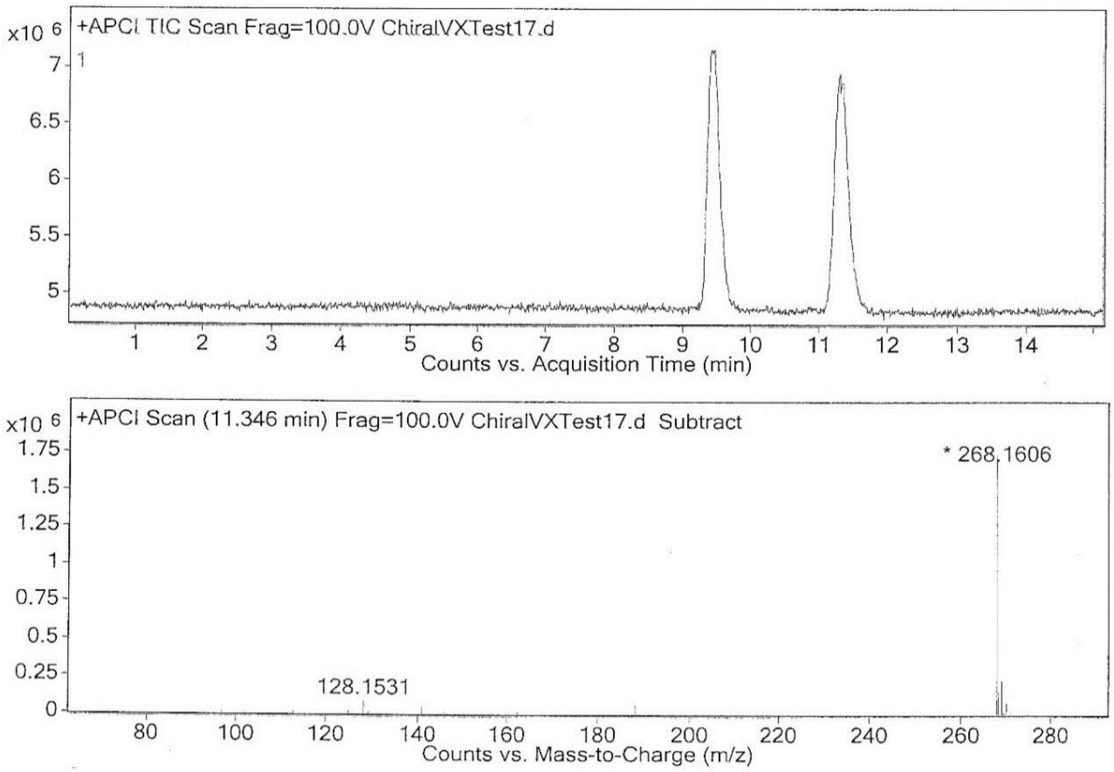

Figure 2. A representative TIC and mass spectrum for VX enantiomers.

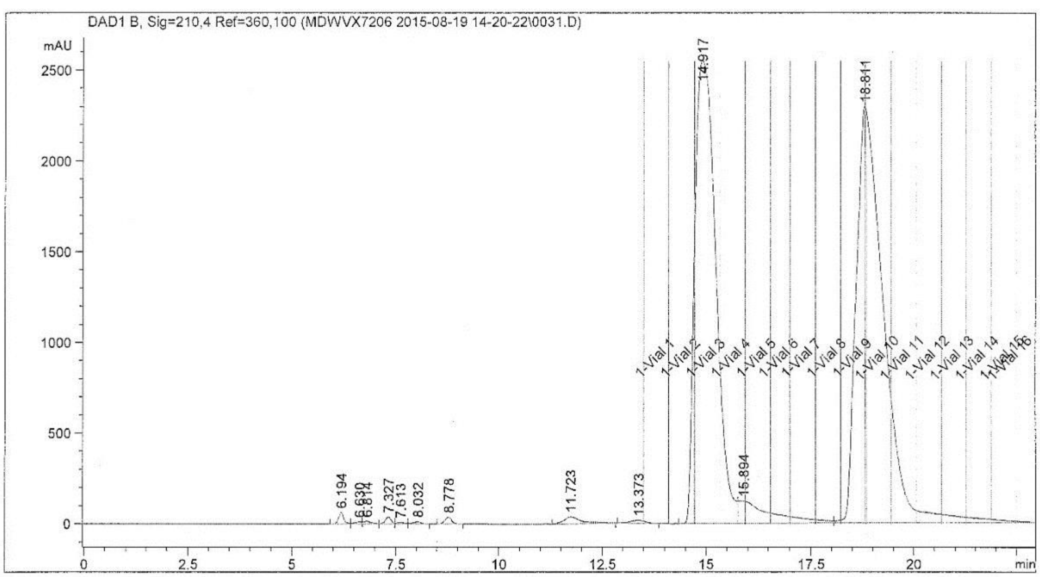

Figure 3. A representative UV chromatogram of VX enantiomers.

When a fragmentor voltage of 100 V was used, the RVX enantiomers eluted at 10.9 and 13.2 min (Figure 4). The mass spectra of the enantiomers were again identical. For the isolation of individual enantiomers, the preparative-scale separation method was the same as that used for VX. A representative UV chromatogram of the RVX enantiomers is shown in Figure 5.

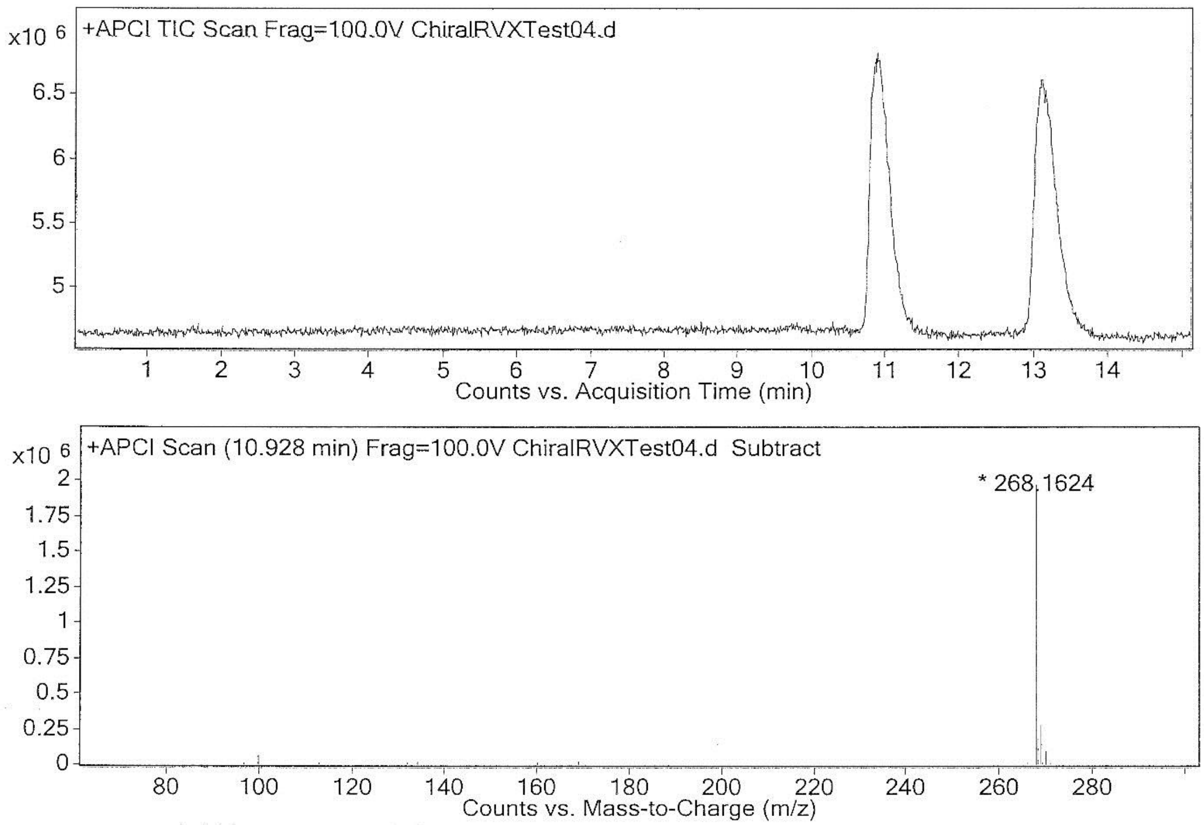

Figure 4. A representative TIC and mass spectrum for RVX enantiomers.

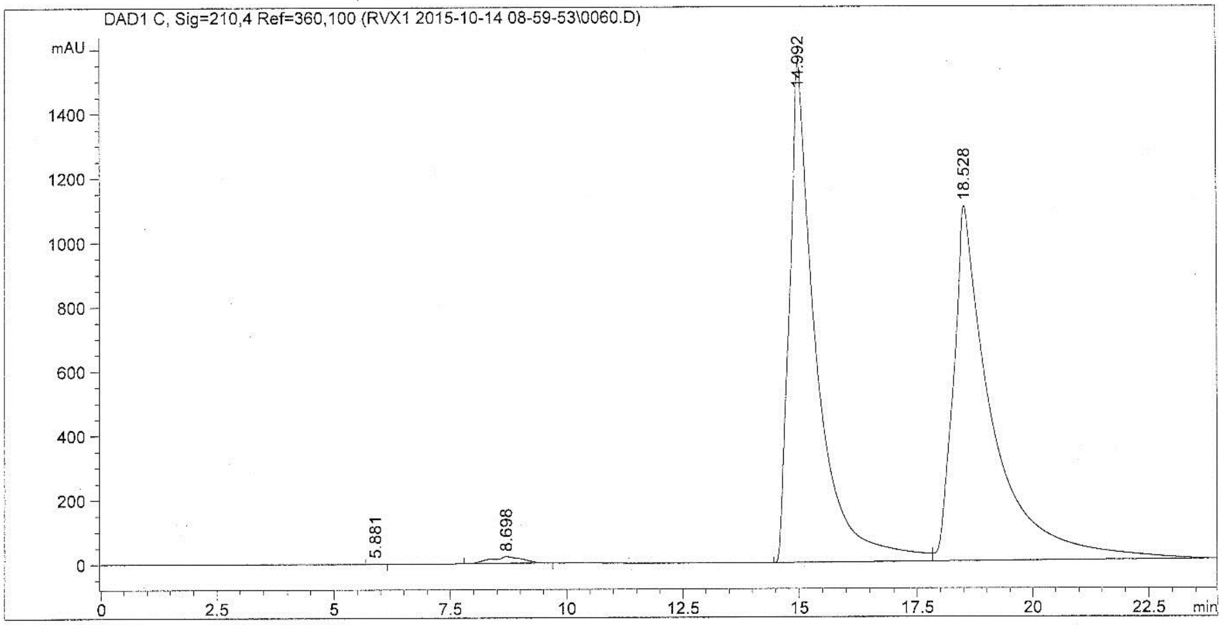

Figure 5. A representative UV chromatogram of RVX enantiomers.

For the enantioselective separation of GB, the enantiomers were baseline-resolved within 15 min using a mobile phase of 95/5 (v/v %) hexane/isopropyl alcohol at a flow rate of 0.6 mL/min. A representative TIC (Figure 6) shows that the GB enantiomers eluted at 12 and 14 min when a fragmentor voltage of 100 V was used. Examination of the mass spectra revealed two peaks, shown at retention times of 6.0 and 9.0 min, that represented impurities in the GB sample. We did not further investigate the impurities in the GB sample. The mass spectra for the GB enantiomers were identical. The mass spectrum for GB shown in Figure 6 exhibits mass ions at *m/z* 98.46 due to loss of a propane group and at *m/z* 158.19 due to $[M+H_2O]^+$. For isolation of the individual enantiomers of GB, the same preparative-scale separation method was used as for VX and RVX.

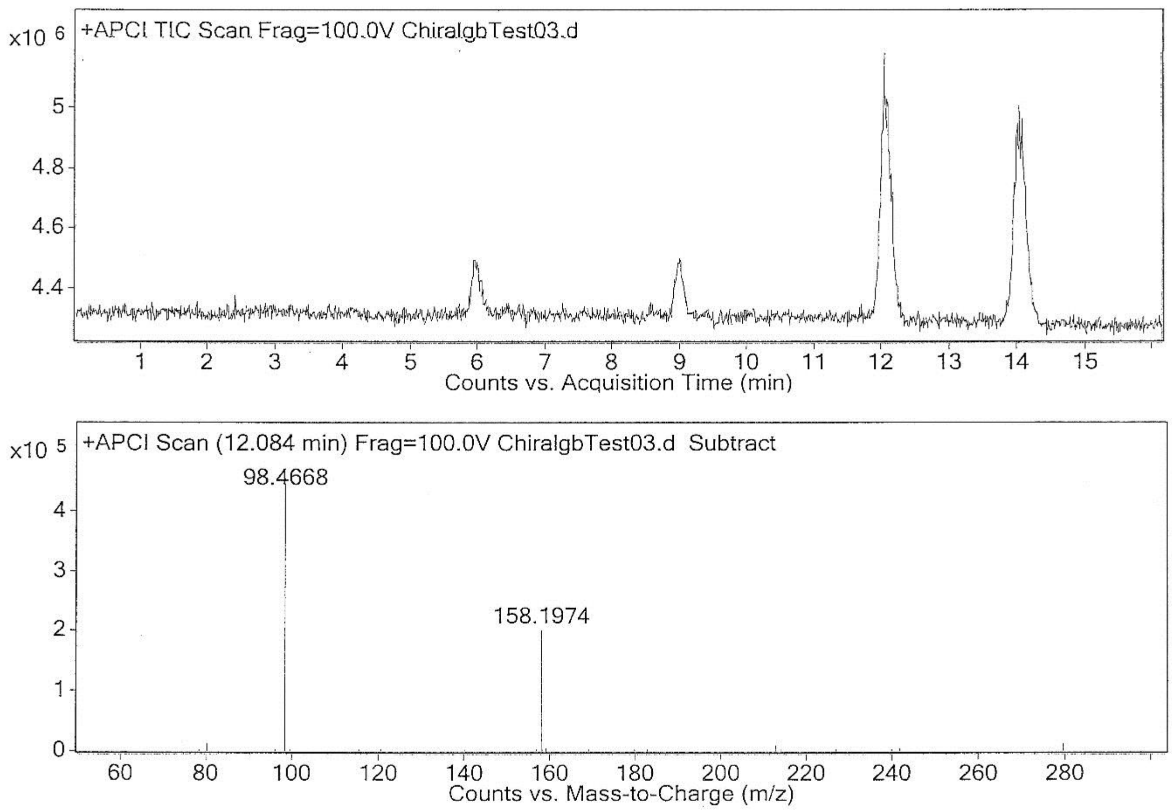

Figure 6. A representative TIC and mass spectrum for GB enantiomers.

4. CONCLUSION

Analytical- and preparative-scale LC methods for the enantioselective separation of VX, RVX, and GB were developed. This report details the separation analysis and results of the study. The separation and isolation methods were easy to use and should be readily accessible for any laboratory. Because of the differing toxicity and acetylcholinesterase inhibition rates between the P(+) and P(–) enantiomers, identification and isolation of each enantiomer is very beneficial for in vitro and in vivo toxicological studies.

LITERATURE CITED

1. Meisenheimer, J.; Lichtenstadt, L. Über optisch-aktive Verbindungen des Phospors. *Berichte der Deutschen Chemischen Gesellschaft* **1911**, *44*, 356–359.

2. Timperley, C.M. *Best Synthetic Methods: Organophosphorus (V) Chemistry,* 1st ed.; Elsevier: London, UK, 2014; Vol. 1.

3. Hall, C.R.; Inch, T.D.; Inns, R.H.; Muir, A.W.; Sellers, D.J.; Smith, A.P. Differences between Some Biological Properties of Enantiomers of Alkyl *S*-Alkyl Methylphosphonothioates. *J. Pharm. Pharmacol.* **1977**, *29*, 574–576.

4. Benschop, H.P.; De Jong, L.P.A. Nerve Agent Stereoisomers: Analysis, Isolation and Toxicology. *Acc. Chem. Res.* **1988**, *21*, 368–374.

5. Reiter, G.; Mikler, J.; Hill, I.; Weatherby, K.; Thiermann, H.; Worek, F. Chromatographic Resolution, Characterisation and Quantification of VX Enantiomers in Hemolysed Swine Blood Samples. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* **2008**, *873*, 86–94.

6. Van den Berg, G.R.; Beck, H.C.; Benschop, H.P. Stereochemical Analysis of the Nerve Agents Soman, Sarin, Tabun, and VX by Proton NMR-Spectroscopy with Optically Active Shift Reagents. *Bull. Environ. Contam. Toxicol.* **1984**, *33*, 505–514.

7. Smith, J.R.; Schlager, J.J. Gas Chromatographic Separation of the Stereoisomers of Organophosphorus Chemical Warfare Agents Using Cyclodextrin Capillary Columns. *J. High Res. Chromatogr.* **1996**, *19*, 151–154.

8. Smith, J.R. Analysis of the Enantiomers of VX Using Normal-Phase Chiral Liquid Chromatography with Atmospheric Pressure Chemical Ionization–Mass Spectrometry. *J. Anal. Toxicol.* **2004**, *28*, 390–392.

Blank

ACRONYMS AND ABBREVIATIONS

APCI	atmospheric pressure chemical ionization
Et(Me)P(O)Ph	ethylmethylphenylphosphine oxide
GB	isopropyl methylphosphonofluoridate, sarin
LC	liquid chromatography
MS	mass spectrometry
OP	organophosphorus
RVX	*S*-(2-(diethylamino)ethyl) *O*-isobutyl-methylphosphonothioate
TIC	total ion chromatogram
VX	*O*-ethyl *S*-[2-(diisopropylamino)ethyl] methylphosphonothioate

DISTRIBUTION LIST

The following organizations were provided with one Adobe portable document format (pdf) version of this report:

U.S. Army Edgewood Chemical
Biological Center (ECBC)
RDCB-DRC-C
ATTN: Bae, S.
Winemiller, M.
Berg, F.

ECBC Technical Library
RDCB-DRB-BL
ATTN: Foppiano, S.
Stein, J.

Defense Technical Information Center
ATTN: DTIC OA

G-3 History Office
U.S. Army RDECOM
ATTN: Smart, J.

Office of the Chief Counsel
AMSRD-CC
ATTN: Upchurch, V.

EDGEWOOD CHEMICAL BIOLOGICAL CENTER

A U.S. Army RDECOM Laboratory